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Research Note

Association Between Alleles of the *Waxy* Gene and Traits of Grain Quality in Philippine Seed Board Rice Varieties

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The association between alleles of the *Waxy* gene, defined by the number of CT repeats on exon 1, and traits of grain quality was carried out using 47 Philippine Seed Board rice varieties. The major alleles of the *Waxy* gene in the set of 47 were $(CT)_{10}$, $(CT)_{11}$, $(CT)_{17}$ and $(CT)_{20}$. Varieties were divided into four clusters based on the metric of each trait of grain quality. $(CT)_{10}$ (27-32% amylose content (AC)) and $(CT)_{11}$ (22-30% AC) were mainly in clusters 3 and 4 (hard texture), and $(CT)_{17}$ (20-24% AC) and $(CT)_{20}$ (18-27% AC) were exclusively in grain quality cluster 1 (soft texture) and 2 (medium texture). $(CT)_n$ associated negatively with AC in this set. Only six $(CT)_{11}$ and one $(CT)_{10}$ rices were in cluster 4 (high-AC low-GT) and had high RVA consistency (final viscosity – trough viscosity) > 200 RVU. $(CT)_{17}$ seemed to be the preferred source of low-intermediate AC in the Philippine rice breeding program, followed by $(CT)_{20}$.

Key Words: apparent amylose content, cooked rice hardness, cluster, CT polymorphism, gelatinization temperature, PSB Rc varieties, RVA, *Waxy* gene

Abbreviations: AC – apparent amylose content, CT – cytosine and thymine, DNA – deoxyribonucleic acid, GBSS I – granule-bound starch synthase I, GQNPC – Grain Quality, Nutrition and Postharvest Centre, GT – gelatinization temperature, IRRI – International Rice Research Institute, PhilRice – Philippine Rice Research Institute, PSB Rc – Philippine Seed Board rice, RVA – Rapid Visco Analyser, RVU – Rapid Visco units

INTRODUCTION

Philippine Seed Board rice (PSB Rc) varieties were classified by Roferos et al. (2006) into four clusters based on physicochemical properties, cooked rice hardness (measured by the Instron Food Tester Model 1140 with extrusion cell) and characteristics of the Rapid Visco Analyser (RVA) curve. Cluster 1 (soft cooked rices) consisted mainly of varieties with both intermediate apparent amylose content (AC) and gelatinization temperature (GT) as well as rices with both low AC and low GT. Cluster 2 (medium cooked rices) consisted of rices either with high AC and intermediate GT or with intermediate AC and low GT. And clusters 3 and 4 (hard cooked rices) consisted solely of rices with high AC and low GT. Varieties in cluster 4 had higher (>200 RVU) RVA consistency (final viscosity – trough viscosity) than cluster 3.

A polymorphic dinucleotide repeat of cytosine and thymine $(CT)_n$ in the 5'-untranslated region of the *Waxy* gene encoding the granule-bound starch synthase I (GBSS I) has been employed in several grain quality laboratories for classification of AC (Bergman et al. 2001; Jayamani et al. 2007). In these laboratories, the association between the number of CT repeats and AC holds very well. The AC in rice associates strongly with the texture of the cooked rice and with the amount the rice paste hardens as it cools from 95 °C to 50 °C in the Amylograph or the RVA (Juliano and Villareal 1993; Bao et al. 2006a). Because these parameters form the definition of the clusters found for a set of 47 PSB Rc varieties (Roferos et al. 2006), the association between the (CT)$_n$ polymorphism and the clustering of PSB Rc varieties was studied using the same set of Philippine rices used by Roferos et al. (2006).

MATERIALS AND METHODS

Seeds from each of the 47 varieties were germinated in the 2005 dry season at the International Rice Research Institute (IRRI). When the seedlings had reached the two-leaf stage, the leaves were harvested for DNA extraction. DNA was extracted using a modified cetyltrimethylammonium bromide method (Allen et al. 2006). The polymerase chain reaction (PCR) was carried out as described previously (Bergman et al. 2001), and the PCR products were separated by polyacrylamide gel electrophoresis exactly as described by Bergman et al. (2001).

RVA characteristics, AC, GT and cooked rice hardness by Instron had been measured previously on the same 47 varieties (Roferos et al. 2006). Validation with the 2005 wet season crop showed the same overall mean AC and GT as the 2002 dry season crop (data not shown). Data were analyzed, using a balanced ANOVA with IRRISTAT for Windows Version 5.0 (2005).

RESULTS AND DISCUSSION

Four main alleles of the *Waxy* gene, defined by the (CT)$_n$, were present in the set of 47 PSB rice varieties: (CT)$_{20}$, (CT)$_{11}$, (CT)$_{17}$ and (CT)$_{10}$ (Table 1). (CT)$_n$ associated negatively with AC in this set. Mean AC was highest for varieties with (CT)$_8$, (CT)$_{10}$ and (CT)$_{11}$, followed by those with (CT)$_{19}$, (CT)$_{20}$ and then the (CT)$_{17}$ allele. The range in AC was narrower for varieties carrying the (CT)$_{10}$ or (CT)$_{17}$ alleles than for those with (CT)$_{11}$ or (CT)$_{20}$ (Table 1). All showed low or intermediate GT (Table 2). GT is not expected to associate with alleles of GBSS I because the activity of soluble starch synthase IIa is known to contribute the most to GT (Umemoto and Aoki 2005; Waters et al. 2006). The wide range of AC for varieties carrying (CT)$_{11}$, (CT)$_{19}$ and (CT)$_{17}$ may reflect the presence of other polymorphic regions in the gene. For example, a polymorphism at the splice site of intron 1 defines two alleles: the Wx^a (indica) and Wx^b (japonica) alleles. Varieties carrying the Wx^a allele have either high or intermediate AC, and those carrying the Wx^b allele are low AC (Jayamani et al. 2007;

Table 1. Comparison of the apparent amylose content of the six microsatellite alleles found in 47 PSB Rc varieties (Roferos et al. 2006).

(CT)$_n$ Allele	Variety (no.)	Amylose Content (%)	
		Range	Mean[a]
8	1	29	29a
10	6	27-32	28ab
11	14	22-30	27ab
17	7	20-24	21d
19	2	21,30	26bc
20	17	18-27	23cd

[a]Means followed by different letters are significantly different (P < 5%). LSD (5%) = 2.7%

Table 2. Relationship of (CT)$_n$ polymorphism in the *Waxy* gene of 47 PSB Rc varieties on grain-quality cluster, AC-GT combination, Instron cooked hardness and RVA viscosity of milled rice (Roferos et al. 2006).

(CT)$_n$ Allele	Cluster Type[a]	AC Type[b]	GT Type[c]		Cooked Hardness (kg cm^{-2})		RVA Viscosity (RVU)				PSB Rc No. (L/IH GT)
							Peak		Consistency		
			L	IH	≤2.5	>2.5	<200	>200	<200	>200	
8	2	H(1)	0	1	0	1	1	0	1	0	0/84
10	2	H(2)	1	1	1	1	1	1	2	0	62/2
10	3	H(3)	3	0	0	3	3	0	3	0	60,86,106/0
10	4	H(1)	1	0	0	1	0	1	0	1	102/0
11	1	I(1)	0	1	1	0	0	1	1	0	0/100
11	2	H(7)	1	6	0	7	0	7	7	0	8/7,36,38,44,68,92
11	4	H(6)	6	0	0	6	0	6	0	6	6,10,46,48,90,96/0
17	1	I(2)	0	2	2	0	0	2	2	0	0/14,54
17	2	I(5)	2	3	5	0	0	5	5	0	34,50/30,56,88
19	1	I(1)	0	1	1	0	0	1	1	0	0/64
19	3	H(1)	1	0	0	1	1	0	1	0	98/0
20	1	I(5)	0	5	5	0	0	5	5	0	0/1,22,58,78,80
20	2	I(8)	2	6	8	0	0	8	8	0	5,12/3,4,28,32,52,82
20	2	H(4)	2	2	2	2	0	4	4	0	24,70/16,66

[a]Cluster 1 soft; cluster 2 medium; cluster 3 and 4 hard (Roferos et al. 2006).
[b]Intermediate – 18–25%; high >25%. Rices with 18–19% AC and intermediate GT were classed as intermediate AC, because low AC rices usually have low GT. Number of samples in parentheses.
[c]Based on alkali spreading value (Little et al. 1958): L = low 6.0–7.0; IH = intermediate-high <6.0.

Chen et al. 2008). The same number of $(CT)_n$ can be present in combination with either polymorphism at the splice site of intron 1 (Jayamani et al. 2007; Chen et al. 2008), which is likely to explain the range of AC for the varieties carrying $(CT)_{11}$, $(CT)_{19}$ and $(CT)_{17}$. The relative AC for selected Portuguese germplasm studied by Jayamani et al. (2007) was higher for varieties with $(CT)_{10}$, $(CT)_{11}$ or $(CT)_{20}$ than for those with $(CT)_{17}$, while $(CT)_{19}$ had lower AC than $(CT)_{20}$ in their study. The relative AC of the 1999 US uniform regional rice nursery (n = 198) was $(CT)_{10}$ and $(CT)_{11}$ > $(CT)_{20}$ > $(CT)_{17}$ (Bergman et al. 2001).

$(CT)_{17}$ and $(CT)_{20}$ were found exclusively in clusters 1 and 2, which are characterized by varieties that are either soft or medium texture when cooked (Roferos et al. 2006). Varieties in cluster 1 have intermediate AC and intermediate GT, and cluster 2 is composed of varieties with intermediate AC and low GT and with high AC and intermediate GT (Roferos et al. 2006). Check varieties with excellent quality, IR64 (intermediate-AC intermediate-GT) and *Sinandomeng* (low-AC low-GT) are in cluster 1 and are $(CT)_{17}$ (data not shown). The popular PSB Rc82 (intermediate-AC intermediate-GT, $(CT)_{20}$) is in cluster 2 (Roferos et al. 2006). The Instron hardness of all $(CT)_{17}$ (all intermediate AC) was < 2.5 kg cm^{-2}. RVA peak viscosity was > 200 RVU for some varieties in clusters 1, 2 and 4, but varieties with either $(CT)_{17}$ and $(CT)_{20}$ had RVA consistency < 200 RVU, consistent with intermediate, rather than high AC (Champagne et al. 1999). Thus, these data and those in the literature (Bergman et al. 2001; Bao et al. 2006b) suggest that $(CT)_{17}$ and $(CT)_{20}$ are associated primarily with intermediate AC and soft to medium hardness on cooking.

$(CT)_{10}$ and $(CT)_{11}$ were mainly found in varieties in either cluster 3 or 4 (hard cooked rice, characterized by high AC and low GT), but $(CT)_{11}$ was also present in cluster 1 and both $(CT)_{11}$ and $(CT)_{10}$ were present in cluster 2 (Table 2). Varieties of high AC with $(CT)_{11}$, $(CT)_{10}$, $(CT)_{19}$ and $(CT)_8$, also showed firmer texture on cooking, but firmness of cooked rice did not associate with any allele of GBSS I defined by the $(CT)_n$ (Table 2). High peak viscosity was found for varieties in cluster 1, 2 and 4, but all high-AC rices in cluster 3 had low peak viscosity (< 200 RVU). High AC rices of $(CT)_{11}$ and $(CT)_{10}$ in cluster 4 had higher consistency (> 200 RVU) than the other high-AC rices, suggesting some differences in starch structure within the group of high AC rices. All seven high AC rices in cluster 4 have low GT. However, not all high-AC low-GT entries had RVA consistency > 200 RVU. In US rices, high-AC rices with superior processing quality have the $(CT)_{11}$ allele but the conventional long-grain rices with intermediate AC have the $(CT)_{20}$ allele (Bergman et al. 2001), all with intermediate GT. Presumably, this association holds because of the narrow germplasm class in the US rices.

Among the high-AC varieties released by IRRI in its early years, intermediate GT entries such as IR5 and IR32 had soft gel consistency and low Amylograph consistency and setback, whereas low GT entries such as IR8 and IR42 had hard gel consistency and high Amylograph consistency and setback (Cagampang et al. 1973; Perez 1979; Juliano et al. 1980, 1987). Such association no longer holds true with current rices. In fact, all three gel consistency types (soft, medium and hard) were reportedly present among both low GT and intermediate GT rices, all with high AC (Villareal et al. 1997), suggesting different regulatory or synthetic pathways leading to gel consistency and GT. Also, all clusters 3 and 4 rices have high AC and low GT, but only cluster 4 rices have higher RVA consistency (Roferos et al. 2006) (Table 2), again suggesting difference in starch structure between the high AC rices in clusters 3 and 4.

$(CT)_{17}$ allele seemed to be the preferred source of low-intermediate AC for the Philippine rice breeding program. The $(CT)_{20}$ allele may also be considered, because good-quality PSB Rc82 has the $(CT)_{20}$ allele, but the AC range for the $(CT)_{20}$ allele is wider (Table 1). $(CT)_{11}$ seemed to be the preferred allele for rices with high and intermediate AC.

CONCLUSION

$(CT)_n$ microsatellite data complement on amylose type in marker-assisted breeding, particularly in cases where the parents have similar AC, but different $(CT)_n$ alleles and where breeding programs aim to capture the quality of one of the parents. $(CT)_n$ associated negatively with AC in this set. The $(CT)_{17}$ allele seemed to be the preferred source of low-intermediate AC in the Philippine rice breeding program, followed by the $(CT)_{20}$ allele.

REFERENCES CITED

ALLEN GC, FLORES-VERGARA MA, KRASNYANSKI S, KUAR S, THOMSON WS. 2006. A modified protocol for rapid DNA isolation from plant tissues using cetyltrimethylammonium bromide. Nat Protocols 1:2320-2325.

BAO J, SHEN S, SUN M, CORKE H. 2006a. Analysis of genotypic diversity in the starch physicochemical properties of nonwaxy rice: apparent amylose content, pasting viscosity and gel texture. Starch/Stärke 58:259-267.

BAO JS, CORKE H, SUN M. 2006b. Microsatellites, single nucleotide polymorphisms and a sequence tagged site in starch-synthesizing genes in relation to starch physicochemical properties in nonwaxy rice (*Oryza sativa* L.). Theor Appl Genet 113:1185-1196.

BERGMAN CJ, DELGADO JT, McCLUNG AM, FJELLSTROM RG. 2001. An improved method for using a microsatellite in rice waxy gene to determine amylose class. Cereal Chem 78:257-260.

CAGAMPANG GB, PEREZ CM, JULIANO BO. 1973. A gel consistency test for eating quality of rice. J Sci Food Agric 24:1589-1594.

CHAMPAGNE ET, BETT KL, VINYARD BT, McCLUNG AM, BARTON II FE, MOLDENHAUER K, LINSCOMBE S, McKENZIE K. 1999. Correlation between cooked rice texture and Rapid Visco Analyser measurements. Cereal Chem 76:764-771.

CHEN MH, BERGMAN CJ, PINSON S, FJELLSTROM R. 2008. *Waxy* gene haplotypes: Associations with apparent amylose content and the effect by the environment in an international germplasm collection. J Cereal Sci 47:536-545.

IRRISTAT for Windows Version 5.0. 2005. Los Baños, Laguna, Philippines: International Rice Research Institute.

JAYAMANI P, NEGRÃO S, BRITES C, OLIVEIRA MM. 2007. Potential of *Waxy* gene microsatellite and single-nucleotide polymorphisms to develop *japonica* varieties with desired amylose level in rice (*Oryza sativa* L.). J Cereal Sci 46:178-186.

JULIANO BO, VILLAREAL CP. 1993. Grain Quality Evaluation of World Rices. Manila: International Rice Research Institute. 205 p.

JULIANO BO, PEREZ CM, BLAKENEY AB, BRECKENRIDGE C, CASTILLO TORO D, CHOUDHURY N, KONGSEREE N, LAIGNELET B, MERCA FE, PAULE CM, WEBB BD. 1980. Report of the international cooperative testing on gel consistency of milled rice. Riso 29:233-237.

JULIANO BO, VILLAREAL RM, PEREZ CM, VILLAREAL CP, TAKEDA Y, HIZUKURI S. 1987. Varietal differences in properties among high amylose rice starches. Starch/Stärke 39:390-393.

LITTLE RR, HILDER GB, DAWSON EH. 1958. Differential effect of dilute alkali on 25 varieties of milled white rice. Cereal Chem 35:111-126.

PEREZ CM. 1979. Gel consistency and viscosity of rice. In: Proceedings of a Workshop on Chemical Aspects of Rice Grain Quality, IRRI, 1978. Los Baños, Laguna, Philippines: International Rice Research Institute. p. 293-302.

ROFEROS LT, FELIX AdR, JULIANO BO. 2006. The search for the grain quality of raw and cooked IR64 milled rice among Philippine Seed Board rice varieties. Philipp Agric Scientist 89:58-70.

UMEMOTO T, AOKI N. 2005. Single-nucleotide polymorphisms in rice *starch synthase IIa* that alter starch gelatinisation and starch association of the enzyme. Functional Plant Biol 32:763-768.

VILLAREAL CP, HIZUKURI S, JULIANO BO. 1997. Amylopectin staling of cooked milled rice and properties of amylopectin and amylose. Cereal Chem 74:163-167.

WATERS DLE, HENRY RJ, REINKE RF, FITZGERALD MA. 2006. Gelatinization temperature of rice explained by polymorphisms in starch synthase. Plant Biotechnol J 4:115-122.